UNITED STATES PATENT APPLICATION

FOR

# SINGLE DETECTOR MULTICOLOR PARTICLE ANALYZER AND METHOD


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## RELATED APPLICATION

This application is a continuation-in-part of co-pending application 10/461,538 filed June 12, 2003.

## BRIEF DESCRIPTION OF THE INVENTION

The present invention relates generally to a multicolor particle analyzer or cytometer and method and more particularly to a multicolor particle analyzer and method which employs a single detector.

## BACKGROUND OF INVENTION

Recent developments in flow cytometry hardware and dye chemistry has made it possible to simultaneously measure as many as ten or more fluorescences and scattered light parameters from cells, beads, molecules, etc. herein referred to as particles. They provide a large amount of novel information, which permits identification and characterization of cell subsets. However, such multicolor prior art systems are complex and expensive. They require multiple optical paths and detectors and complex control circuits. They are not suitable for portable use, point of care use or battlefield use.

Conventional flow cytometers require hydrodynamic sheath flow to align the particles in a single line in the laser probe volume. The hydrodynamic focusing accelerates the sample and particles and requires relatively large volumes of sample and sheath fluid to carry out an analysis. Typically for sample volume flow rates of 1 $\mu L$ /s and exit velocities of 25-50mm/s the particle velocities reach 10m/s when they cross the probe volume. For a probe beam laser width of 20 microns, the particle time of flight through the probe volume is 2 microseconds. Since the particle is present in the probe volume for only a short time, a detection system of relatively large bandwidth is required for each color thus leading to complex, expensive and bulky systems.

## OBJECT AND SUMMARY OF THE PRESENT INVENTION

It is an object of the present invention to provide a simple, relatively inexpensive multicolor particle detection system and method.

It is a further object of the present invention to provide a multicolor particle detection system and method, which employs a single detector.

It is a further object of the present invention to provide a multicolor particle detection system in which multiple reading of the distinct multicolor fluorescences of each particle is obtained during the time of flight of the particle through a probe volume in a capillary and therefore enables the reconstruction of the fluorescent traces of each particle using a single detector.

It is a further object of the present invention to provide a multicolor particle analyzer which employs an acousto-optical bandpass filter to repetitively sample fluorescent light at different wavelengths (color) in sequence as particles pass through the probe volume in a capillary.

The foregoing and other objects of the invention are achieved by a system in which the sample particles each of which fluoresce at one or multiple distinct wavelengths flow slowly through a capillary tube past a detection or probe volume where the particles are excited by a light beam and fluoresce at characteristic wavelengths. The fluorescent light is applied to an acousto-optical bandpass filter whose pass band repetitively changes wavelength multiple times as a particle passes through the probe volume and whose output is applied to a single detector. The output of the detector is reconstructed to provide a characteristic fluorescent trace for each fluorescent wavelength of the particle.

DESCRIPTION OF THE FIGURES

The foregoing and other objects of the invention will be more clearly understood from the following description when read in connection with the accompanying drawings of which:

Figure 1 is a schematic diagram of the multicolor particle analyzing system of the present invention;

Figure 2 is an enlarged view of a portion of the interior of the capillary shown in Figure 1;

Figure 3 is a scatter trace for a particle flowing through the analyzing volume;

Figure 4 shows the traces obtained from a particle tagged to fluoresce at four different wavelengths as it flows through the detection region;

Figure 5 shows flow through a capillary independent of whether the sample is aspirated or pumped;

Figure 6 shows the light wavelength pass bands of the acousto-optic filter as a function of the RF voltage frequency; and

Figure 7 show stepping the RF frequency to obtain sequential pass bands; and

Figure 8 is a schematic block diagram of a system for controlling the acquisition and detection of fluorescent light from a particle traveling through the analyzing region.

DESCRIPTION OF PREFERRED EMBODIMENTS

A particle analyzing apparatus suitable for carrying out the invention is shown in Figure 1. Briefly, a particle suspension 11 containing particles (as used herein "particles include cells, molecules, beads, etc.) to be analyzed flows through a capillary 12 as shown in Figures 1 and 5. Preferably the capillary is a square capillary. The sample with suspended particles is aspirated or drawn through the capillary by a pump 13. A laser or other suitable light source projects a beam 14 through the capillary to define an analyzing volume 16. The particle suspension flows through analyzing volume 16 with the cells singulated. Cells or particles, or beads which have been tagged with one or more dyes which fluoresce at distinct wavelengths are excited by the light beam 14 as they pass through the analyzing volume and emit light at the distinctive wavelengths. Scattered light is gathered by a lens system 17 and is focused onto detector 18 which provides a count of all particles which have traversed the volume whether labeled or not and information regarding their size. Cells which have been tagged or labeled with a distinct or characteristic dye or which naturally fluoresce emit light at the dye's corresponding wavelength. The light emitted by each tagged particles is gathered by a lens system 21 and applied to an acousto-optic tunable filter 22. The acousto-optic filter is driven by an integral transducer 23. The acoustic-optic filter is a solid-state electronically tunable band pass filter which uses acousto-optic interaction inside an anisotropic medium. It allows the user to select and pass or transmit a single tunable wavelength from the incoming light. The RF frequency applied to the transducer 23 controls the wavelength of the fluorescent light that is transmitted. The acousto-optic filter rapidly, sequentially and repetitively shifts the light wavelength which it passes so that as particles, cells or beads traverse the detection volume and emit fluorescent light at one or more characteristic wavelengths. The fluorescent light is periodically sampled and applied to the detector 24 a number of times for each wavelength during the transit time of that particle through the analyzing volume. Since the pass band is repetitively shifted and the process repeated during

the transit time of a particle, the detector provides output pulses corresponding to the intensity of emitted fluorescent light at the characteristic wavelength of each label at the sampling intervals. As will be presently described, the signals can be reconstructed to provide a fluorescence trace for the particle. Since the wavelength pass band is repetitively shifted during passage of a particle the multiple wavelengths are sampled and detected whereby to provide multiple fluorescent traces for each particle.

Referring particularly to Figure 2, as a particle passes through the detection volume 16 the fluorescence is periodically detected when the wavelength pass band corresponds to the fluorescent wavelength of the particle. Assume the laser beam 14 has thickness of 20 $\mu$m and the capillary dimensions a = b = 0.1mm, and the volume of sample from the inlet 26 to the detection volume is 200 nanoliters and the probe volume is .2 nanoliters. Assume further that the flow rate is 1 microliter per second, then the particle velocity is 100mm/s and the transit time through the detection volume is 200 $\mu s$. Assume that the acousto-optic filter can shift its pass band in 10 microseconds or less, this would result in sampling the fluorescent emission of each particle in a four color system about 5 times. It is apparent that if the acousto-optic filter can shift at a greater frequency, more samples can be taken or, in the alternative, a larger number of fluorescence colors can be detected and provide sufficient sampling points to reconstruct the fluorescence trace. It is also to be of particular note that the system requires small volumes of sample. The system is ideally suited for cell subset analysis wherein only small volumes of blood are available. This permits cell analysis which were, heretofore, difficult to perform because of the small volume of blood available, for example from infants, small animal species, mice, rats and other living organisms. The ability to analyze small volumes of blood from a living organism will allow characterization of blood cell populations without sacrificing the animals and will permit longitudinal studies where samples can be taken from a single animal at periodic intervals.

For example, consider a sample with particles each of which have been tagged or naturally fluoresce at different wavelengths, say 532nm, 580nm, 675nm and 700nm, passing through the detection volume. The traces shown in Figure 4 show the sampling points for each of four particles obtained by rapidly, sequentially, and repetitively detecting the fluorescence and using the sampling points to construct the traces for the four particles. It is seen that the

amplitude of the signal increases as the particle travels into the detection volume and decreases as the particle leaves the detection volume. This provides a peak signal for each color.

The acoustic-optic tunable filter passes light at a wavelength or frequency which is determined by the RF frequency of the drive voltage applied to the transducer 23. Figure 6 is a schematic plot of pass wavelength as a function of the RF frequency applied to the transducer 23. If the RF frequency is periodically and repeatedly increased as shown in Figure 7 the pass bands are also periodically and repetitively decreased in wavelength. As an example, the wavelengths of the pass bands for the above-noted wavelengths is illustrated in Figure 7. Should there be particles which emit at other wavelengths the RF frequency could be adjusted to drive the AO filter at the appropriate pass band wavelength. In other words, the AO filter could be tuned to the maximum spectral emission range of the particles. Although an AO filter is preferred other tunable filters could be used.

Referring to Figure 7 a suitable circuit for controlling the tunable filter and constructing the traces for each particle is schematically shown. The circuit includes a source of timing pulses 31 which time the repetition of the RF frequency ramps 32. The transducer 23 is driven by the RF voltage ramps and drives the tunable filter 22 which receives the fluorescent light and selectively passes it to the detector 24. The detector provides output pulses 33 having an amplitude corresponding to that of the impinging light. A processor 34 synchronized by the timer receives the voltage pulses and reconstructs the traces, Figure 4, for each wavelength. A peak detector (not shown) can provide a signal pulse representing the amplitude. The peak from a number of particles can then be plotted as a function of wavelength.

Thus there has been provided a particle analyzer using a single optical arrangement and detector to simultaneously measure a large number of fluorescent light parameters and provide information which permits characterization of particles which fluoresce at different wavelengths.